MCLAB

DNA Homopolymeric Tailing SuperMix
User Manual

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Cat # (NGMA-100, NGMA-200, NGMT-100, NGMT-200, NGMC-100, NGMC-200, NGMG-100, NGMG-200)

Description

DNA Homopolymeric Tailing SuperMix provides qualified reagents for the addition of homopolymer tails to the 3' ends of DNA with terminal deoxynucleotidyl transferase (TdT). Under optimized assay condition, approximately average of 30~70nt oligo(dA) and oligo(dT) or 15~45nt oligo(dC) and oligo(dG) could be added to the target substrate.

TdT is a template-independent DNA polymerase that catalyzes the repetitive addition of deoxynucleotides to the 3' hydroxyl terminus of DNA molecules. The enzyme was generated from an E. coli strain that carries the cloned TdT gene from calf thymus with selected mutations. Protruding, recessed or blunt-ended double or single-stranded DNA molecules serve as a substrate for TdT. The addition of dNTPs to 3′-overhanging ends is more efficient than with 3'-recessed or blunt ends. TdT incorporates dATP and dTTP with higher efficiency than dCTP and dGTP. The addition of Co2+ stimulates the tailing of the 3'-ends of DNA fragments, even applicable for incorporating ribonucleotides and modified nucleotides (e.g., fluorescein-, biotin-, aminoallyl-labeled nucleotides and dideoxynucleotides).

Protocol

We recommend assembling reactions on ice from pre-chilled components. This protocol is for a reaction size of 10 µL. The reaction size may be adjusted as desired.

1. Set up reaction as below.

Amount	Description	Final Concentration
5 µL	2X SuperMix	1X
X µL	DNA termini	1µM
X µL	Nuclease free water	N/A
10 µL	Total volume	

2. Incubate at 37°C for 15 to 45 minutes, depends tail length expected.

3. Inactivate the TdT and stop the reaction by heating to 70°C for 10 minutes or directly add 0.5ul 0.5mM EDTA.

Note

Input quantity of DNA substrate is critical to the tail length of final product. Reaction time could be adjusted according to expected tail length.

Repeated freeze-thaw cycles may reduce SuperMix performance or TdT enzyme activity.

Due to the presence of CoCl2, the tailing reaction mixture is incompatible with downstream applications. It is necessary to remove CoCl2 by spin column or phenol/chloroform extraction and subsequent ethanol precipitation.

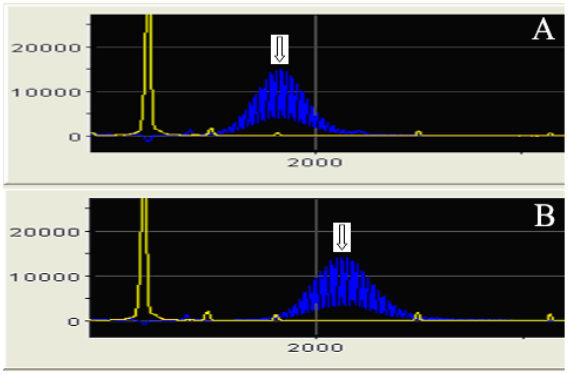

Figure 1. Fragment analysis through 3730 xl DNA Analyzer shows an oligo(dA) tail was added to 5' fluorescently labeled single strand DNA oligo using DNA Homopolymeric Tailing Supermix (dA). Arrow: Tailing reaction final product. A: Fifteen minutes at 37°C reaction product with a 50 nt average length of homopolymer dA tail. B: Thirty minutes at 37°C reaction product with a 65 nt average length of homopolymer dA tail.

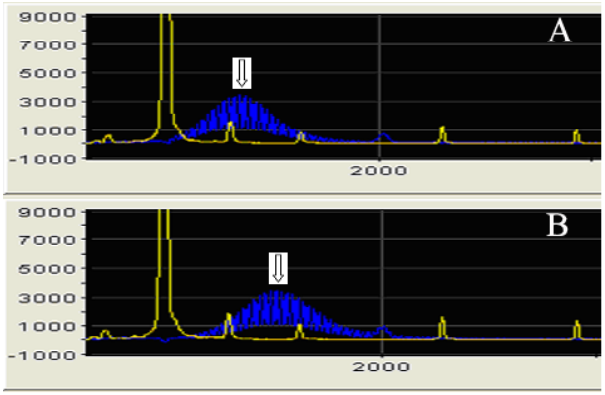

Figure 2. Fragment analysis through 3730 xl DNA Analyzer shows an oligo(dC) tail was added to 5' fluorescently labeled single strand DNA oligo using DNA Homopolymeric Tailing Supermix (dC). Arrow: Tailing reaction final product. A: Tweenty minutes at 37°C reaction product with a 38 nt average length of homopolymer dC tail. B: Forty minutes at 37°C reaction product with a 45 nt average length of homopolymer dC tail.

Reference

1. F. J. Bollum, Terminal deoxynucleotidyl transferase, The Enzymes, the third edition (Boyer, P.D., ed.3, Academic Press, New York, vol.10, 1974) pp. 145-171.

2. G. R. Deng, R. Wu, Terminal transferase: Use in the tailing of DNA and for in vitro mutagenesis. Meth. Enzymol. 100, 96-116 (1983).